**Background**

Formalin test is a widely used method to measure analgesic effect. Injection of formalin into one hindpaw of rats elicits behavioural signs of pain, such as licking of the paw. Our goal was to replace the traditional and time consuming observational work of humans by an automated method. A special new algorithm was designed for LABORAS™, an automated system to measure and analyse rodent behaviour, allowing parallel observation of 4-6 rats.

First, we compared the LABORAS™ measurement and observers' scores and then we determined ED₅₀ of several well-known compounds.

**Methods**

Rats were pretreated orally just before formalin injection with four different treatment (at least 3 animals in each group). After pretreatment all rats were injected with 50 μl 2.5% formalin in the right hind limb dorsally and observed by the LABORAS™ equipment and, at the same time, recorded on video from the side of cage for at least 30 minutes following the injection. To improve the visibility of rat when facing away from the camera, two mirrors were placed beside and behind the cage. The duration of experiments was between 30 and 60 minutes.

For pharmacological validation we measured the time spent with licking after the administration of different compounds with known analgesic effect belonging to different classes regarding their mechanism of action. Formalin injection into hindpaw produces a characteristic biphasic curve of pain related licking behavior. First phase (0-10 min) represent acute pain, while second phase (15-25 min) reflects peripheral activity.

**Statistical analysis**

Data are presented as means ± SEM. Statistical comparisons between mean of observers and LABORAS™ data were performed using unpaired t-test at each interval (Instat, GraphPad, San Diego, USA). Significance was considered at 0.05 level (* indicates p<0.05).

In pharmacological validation analysis of variance (ANOVA) with post hoc Tukey-test (Instat, GraphPad, San Diego, USA) was used to compare the effect of different drugs between the groups. Asterisks (‘*’ and ‘**’) indicate p<0.05, p<0.01 and p<0.001, respectively.

**Results I. Comparison between scores**

**Results II. Pharmacological validation**

**Discussion**

Evaluating the LABORAS™ formalin result and observers' scores data we can find that the difference between observers and laboras is not higher than it can be measured between observers alone. The highest difference can be found at the descending part of the 2nd phase. This is the part of pain related licking behavior where animals are licking their paw frequently for short time.

There is a possibility of a small systemic error made by LABORAS™ or by observers, as well. Probably, the longer reaction time of humans can make such difference. The difference is small and seems not to disturb reliable assessment of inhibition by a drug which was the main goal of the test.

**Conclusion**

No significant difference could be observed between LABORAS™ new formalin software and observers' scores. Furthermore, we measured different compounds and calculated ED₅₀ in 1st and 2nd phases in rat formalin test. The calculated ED₅₀ values measured with LABORAS™ formalin test are in agreement with previous findings.

Our conclusion is that LABORAS™ new formalin software provides a fast and reliable measurement to assess the effects of analgesic compounds.